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A STUDY OF ULTRACENTRIFUGATION OF EUGLENA GRACILIS KLEBS 1883

by

Truman Bertrum Carlson

Approved by Committee

Leland J. Johnson
Chairman

Rodney A. Rogers

R. R. Hann

Sarah T. Canfield
Dean of the Graduate Division

A STUDY OF ULTRACENTRIFUGATION OF EUGLENA GRACILIS KLEBS 1883

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Truman Bertrum Carlson

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CHAPTER I

INTRODUCTION

The development of the space program opened a vast new field of research for the scientist. Of particular interest to the biologist are the many factors which exert an influence on living organisms, with emphasis on those which have a direct effect upon the human body. One such factor is the effect of centrifugal forces.

With the use of the ultracentrifuge, it is possible to test the effect of these forces on living cells. Previous investigations in this field have noted stratification of organelles in cells. Little attention has been given to the recuperative powers of the cell. In an attempt to determine this, Euglena gracilis Klebs, 1883 was placed in an air-driven centrifuge at a force near 125,000 times gravity to distort the position of the cellular components.

It has been the purpose of this investigation to study (1) the time required for redistribution of the various organelles of Euglena gracilis Klebs, 1883 after centrifugation; and (2) what sequence, if any, was followed in the redistribution of organelles.

CHAPTER II

REVIEW OF LITERATURE

The centrifuge has been a useful aid in the study of cell components. Due to variations in specific gravity of cell organelles, it has been possible to bring about their stratification. This has led to the identification of previously unknown structures and increased knowledge of others.

The potential value of the use of the centrifuge was recognized as far back as the nineteenth century. Mottier reported on the "Effect of Centrifugal Force Upon the Cell" in 1899. He used an ordinary milk centrifuge driven by a gas motor which was capable of producing a force from 1700 to 1930 times the force of gravity. Redistribution of cell contents was noted in Cladophora and various other algae.[1] In 1909, McClendon studied the effects of the centrifuge on protozoans.[2] Yancey used the Sharples Super Centrifuge, producing 21,000 to 32,000 times the force of gravity, in

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[1] D. M. Mottier, "Effect of Centrifugal Force Upon the Cell," Annals of Botany, XIII (September 1899), 325-57.

[2] J. F. McClendon, "Protozoan Studies," Journal of Experimental Zoology, VI (February 1909), 269.

his work on Paramecium in 1931.[1] However, it was not until the modification of the high speed, air-driven ultracentrifuge by J. W. Beams and associates that the full potential of centrifugation was realized. Previous devices used were limited in speed by moving parts and the resulting build-up of heat. The ultracentrifuge developed by Beams and his associates is a relatively simple device consisting of an air-driven rotor spinning on a cushion of air.[2] In his studies of the air-driven ultracentrifuge, H. W. Beams found that the "temperature does not vary in the centrifuge chamber over 2 or 3 degrees from that of the atmosphere; this is not sufficient to be an important factor in general biological work."[3]

Due to the variation in the specific gravity of various cell components, ultracentrifugation of cells will result in the various components collecting in layers from

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[1]Patrick H. Yancey, "Effect of Super-centrifuging on Fission in Paramecium," Proceedings of the Society for Experimental Biology and Medicine, XXVIII (June 1931), 877.

[2]J. W. Beams, "An Apparatus for Obtaining High Speeds of Rotation," The Review of Scientific Instruments, I (November 1930), 667-71; J. W. Beams, A. W. Weed, and E. G. Pickles, "The Ultracentrifuge," Science, LXXVIII (October 13, 1933), 338-40.

[3]Harold W. Beams, "The Air Turbine Ultracentrifuge, Together With Some Results Upon Ultracentrifuging the Eggs of Fucus serratus," Journal of the Marine Biological Association of The United Kingdom, XXI (March 1937), 585.

the centrifugal pole to the centripetal pole. Beams, Gatenby, and Muliyil found the Golgi apparatus in the upper layer, mitochondria in the lower layer, and clear cytoplasm in between in their work on the spermatocytes of Helix aspersa.[1] In a study of Fucus serratus eggs, Beams found the visible inclusions stratified according to their relative specific gravity.[2] In their studies of the effect of ultracentrifugation on paramecia, King and Beams found, in order from centrifugal to centripetal pole: "crystals, layer of fluid, micronucleus and macronoclear chromatin, food vacuoles and neutral red inclusions, achromatic matrix of the macronucleus, endoplasm with large clear alveoli, and fat."[3] Beams and King noted the presence of a layer they thought to be mitochondria in their investigation of centrifuged Ascaris eggs.[4] In his investigation of Euglena rubra Hardy,

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[1]H. W. Beams, J. Bronte Gatenby, and J. A. Muliyil, "Ultracentrifuging the Spermatocytes of Helix aspersa," Quarterly Journal of Microscopical Science, LXXVIII (February 1936), 394.

[2]Harold W. Beams, loc. cit.

[3]R. L. King and H. W. Beams, "The Effect of Ultracentrifuging on Paramecium, With Special Reference to Recovery and Macronuclear Reorganization," Journal of Morphology, LXI (June 1937), 27.

[4]H. W. Beams and R. L. King, "The Suppression of Cleavage in Ascaris Eggs by Ultracentrifuging," Biological Bulletin, LXXIII (August 1937), 101.

1911, Johnson found the paramylum bodies at the centrifugal end. Lying centripetal to these were the chloroplasts, nucleus, haematochrome, and mitochondria.[1] Beams also noted stratification in the human erythrocyte[2] and in the Elodea.[3]

Although various investigators have studied cell structure with the use of the ultracentrifuge, few have studied the recovery of the cell to any great extent. Two major factors will cause variation in the effect of ultracentrifugation on the cell and on cell recovery. These are the force used and the length of time exposed to the force. Variation of one or the other or both will bring about differing results. Also, different cells will be affected in different ways. In his work with Fucus serratus, Beams found that eggs which had been exposed to 150,000 G for one-half hour apparently developed normally.[4] Similar results

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[1]Leland P. Johnson, "A Study of Euglena rubra Hardy 1911," Transactions of the American Microscopical Society, LVIII (January 1939), 43.

[2]Harold W. Beams, "Stratification of the Human Erythrocyte by Ultracentrifuging," Anatomical Record, XCVII (March 1947), 380.

[3]Harold W. Beams, "Some Effects of Centrifuging Upon Protoplasmic Streaming in Elodea," Biological Bulletin, XCVI (June 1949), 247.

[4]Harold W. Beams, "The Air Turbine Ultracentrifuge, Together With Some Results Upon Ultracentrifuging the Eggs of Fucus serratus," Journal of the Marine Biological Association of The United Kingdom, XXI (March 1937), 585.

were found by Beams and King in their work with Ascaris eggs. "Uncleaved eggs centrifuged at 400,000 times gravity for 30 minutes are not killed, and, if the stratified materials are redistributed before cleavage takes place, they apparently develop normally."[1] King and Beams made a rather thorough investigation of the effects of ultracentrifugation on Paramecium. They found that Paramecium caudatum frequently survived centrifuging for five minutes at 21,000 times gravity, recovered their normal shape, and often underwent fission. However, exposure for ten minutes or longer usually resulted in death of the organism.[2] Beams also noted recovery in cells of Elodea.[3]

In 1936, Patten and Beams reported on investigations carried out on some free-living flagellates, with particular emphasis on a species of Euglena. The euglenae were centrifuged in an air-driven ultracentrifuge at 100,000 times

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[1]H. W. Beams and R. L. King, "The Suppression of Cleavage in Ascaris Eggs by Ultracentrifuging," Biological Bulletin, LXXIII (August 1937), 110.

[2]R. L. King and H. W. Beams, "The Effect of Ultracentrifuging on Paramecium, With Special Reference to Recovery and Macronuclear Reorganization," Journal of Morphology, LXI (June 1937), 30.

[3]Harold W. Beams, "Some Effects of Centrifuging Upon Protoplasmic Streaming in Elodea," Biological Bulletin, XCVI (June 1949), 255.

gravity for two and one-half to three minutes.[1] They observed very definite stratification of the organelles. The paramylum bodies and "neutral red bodies" were found at the centrifugal pole; next came the chloroplasts and nucleus; and at the centripetal end were the cytoplasm and tiny particles they considered to be mitochondria.[2] They reported that:

> When examined rapidly after centrifuging, most of the organisms have contracted into a more or less oval shape and are non-motile. Those that are in the fully elongated condition throughout generally do not show such clear-cut stratification. However, restoration of motility soon occurs, followed later by redistribution of the components, so that after a time (6 hours--overnight) one can no longer perceive that the organisms have been exposed to the centrifugal force.[3]

In his study of Euglena rubra Hardy, 1911, Johnson observed similar results regarding relative positions of cell components after ultracentrifuging at 150,000 times gravity for two minutes. Redistribution was usually accomplished in fifteen minutes.[4]

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[1]Ruth Patten and Harold W. Beams, "Observations on the Effect of the Ultracentrifuge on Some Free-living Flagellates," Quarterly Journal of Microscopical Science, LXXVIII (July 1936), 617.

[2]Ibid., 631.

[3]Ibid., 619.

[4]Leland P. Johnson, "A Study of Euglena rubra Hardy 1911," Transactions of the American Microscopical Society, LVIII (January 1939), 43-44.

Morris, Beischer, and Zarriello used a Spinco ultra-centrifuge (Model E) in experimenting on several organisms, including Euglena gracilis. About half of the euglenae which were centrifuged at 212,000 times the force of gravity for four hours survived. No unusual behavior was observed during the post-exposure period.[1]

It is evident that Euglena can survive exposure to centrifugal force. While investigators have varied the amount of force to which euglenae have been exposed, few have reported on the variation in exposure time.

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[1]David P. Morris, Jr., Dietrich E. Beischer, and Jerry J. Zarriello, "Studies on the G Tolerance of Invertebrates and Small Vertebrates While Immersed," Journal of Aviation Medicine, XXIX (June 1958), 438.

CHAPTER III

MATERIALS AND METHOD

The organism used in this study was Euglena gracilis Klebs, 1883. A pure culture of the organism was secured from the Drake University Biology Department. Periodically, new cultures were started so as to maintain an adequate supply of organisms for experimental purposes.

The Euglena were cultured in test tubes (Kimax--screw type, 20 by 150 mm.) in fifteen milliliters of Euglena Broth secured from Difco Laboratories, Detroit, Michigan. To maintain bacteria-free cultures, the media and glassware used in culturing the organism were sterilized in a pressure cooker at fifteen pounds pressure for fifteen minutes. Transfers from the stock culture were carried out in a specially constructed box to protect the media from air-borne contaminants. The transfers were made with a sterile inoculating loop. These cultures were grown at a temperature of twenty-seven degrees centigrade, which falls within the range suggested by Bach.[1] Environmental factors can greatly

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[1]Michael K. Bach, "Mass Culture of Euglena gracilis," Journal of Protozoology, VII (February 1960), 50-51.

affect growth and chlorophyll synthesis in Euglena.[1]

The organelles observed in this study included the nucleus, chromatophores, paramylum bodies, mitochondria, and carotinoid granules of the stigma. To facilitate the identification of the mitochondria in living specimens, the vital stain, Janus Green B (Fisher Scientific Company, Pittsburg, Pennsylvania) was used. The Euglena were stained with a 1:100,000 solution for a minimum of two hours. This was accomplished by adding one milliliter of a 1:10,000 solution of the stain to nine milliliters of the culture medium. The identification of the remaining organelles was sufficiently clear so no further staining was felt necessary.

Four milliliters of the culture were placed in an air-driven ultracentrifuge and subjected to a force near 125,000 times gravity for a period of three minutes. Equal amounts of the culture were subjected to the same force for periods of fifteen minutes, thirty minutes, forty-five minutes, one hour, and one and one-half hours. The euglenae were also centrifuged for periods of time through three and one-fourth hours in preliminary studies.

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[1]J. J. Wolken, A. D. Mellon, and C. L. Greenblatt, "Environmental Factors Affecting Growth and Chlorophyll Synthesis in Euglena. I. Physical and Chemical. II. The Effectiveness of the Spectrum for Chlorophyll Synthesis," Journal of Protozoology, II (August 1955), 95.

Four observations were made: (1) immediately after removal from the centrifuge, (2) thirty minutes after removal, (3) one hour after removal, and (4) two hours after removal. In the first observation, the degree of stratification and relative positions of the various layers of organelles was noted. In the succeeding observations, the degree of redistribution of organelles and the sequence followed was observed. Observations were made with an A. O. Spencer compound microscope (10x eyepiece; 16 mm., 4 mm., and 1.8 mm. oil immersion objectives).

Permanent slides were made at the same time intervals as microscopic examination of the live specimens were made. The organisms were fixed in Schaudinn's fluid.[1] Two milliliters of fixative were added to a test tube (12 by 75 mm.) containing one-half milliliter of the centrifuged culture for a period of thirty minutes. The Euglena were washed in 35 per cent ethyl alcohol for thirty minutes. To remove the mercury, the organisms were placed in a solution of 50 per cent alcohol to which a few drops of a saturated solution of iodine had been added. The fixed organisms remained in this solution one hour. This was followed by a fifteen minute wash with 35 per cent alcohol and two successive

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[1]Richard R. Kudo, Protozoology (third edition; Springfield, Illinois: Charles C. Thomas, 1946), p. 725.

fifteen minute washes in distilled water.

A modified form of Heidenhain's iron alum haematoxylin[1] stain was used. The organisms were placed in four per cent iron alum for twenty-four hours. This was followed by two successive five minute washes with distilled water. The Euglena were then stained with haematoxylin for twenty-four hours. After a five minute wash with distilled water, the organisms were placed in two per cent iron alum. The clearing process was observed under the compound microscope and halted at the appropriate time with a tap water wash of five minutes.

The standard ethyl alcohol dehydration series[2] was followed except for additional steps of absolute alcohol and toluene. All washes were continued for a period of ten minutes, except the second absolute alcohol and toluene washes, which were fifteen minutes.

To transfer from one solution to the next, the Euglena were concentrated by centrifuging in a low-speed centrifuge for two minutes. The top two milliliters of the solution were removed with a pipette and discarded. Two milliliters

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[1] Ibid., p. 727.

[2] Peter Gray, Handbook of Basic Microtechnique (New York: McGraw-Hill Book Company, Inc., 1958), p. 98.

of the next wash were added. The test tube was shaken vigorously and allowed to stand for the required time period.

A drop of the Euglena preparation was then mounted in H·S·R (Harleco Synthetic Resin) secured from Hartman-Leddon Company, Philadelphia, Pennsylvania.

CHAPTER IV

RESULTS AND INTERPRETATION OF DATA

The normal distribution of organelles in Euglena gracilis were observed before the results of centrifugation and redistribution of organelles were studied. The trophozoite of Euglena gracilis averaged about fifty μ long by fifteen μ in diameter. There was a single nucleus lying in the center or slightly posterior of the center of the cell. It appeared as a clear or transparent sphere. There were approximately a dozen elongated discoid chloroplasts evenly distributed throughout the organism. The margin of these structures was usually obscured by the numerous annular paramylum bodies. A cytopharynx extended from the anterior end of the organism into a spherical reservoir. Protruding externally from the cytopharynx was a single flagellum. Adjacent to the reservoir was an orange-red stigma, composed of many small granules. Numerous spherical-shaped mitochondria were observed evenly distributed throughout the cell. Intravital staining with Janus Green B was ineffective in the present strain of Euglena gracilis. In addition to this elongated form, the euglenae were found in a contracted,

inactive form. In this state, the shape was ovoid.[1]

Upon exposure to centrifugation, the euglenae generally were contracted into the inactive state. Whereas seventy per cent were in the elongated shape prior to centrifugation, only thirty-two per cent were observed in this shape immediately after centrifuging for three minutes, and nineteen per cent after centrifuging for fifteen minutes, as shown in Table I. The percentage in the elongated stage was fifty-one per cent after thirty minutes, fifty-four per cent after one hour, and fifty-five per cent after two hours in organisms centrifuged for three minutes. Corresponding percentages for organisms centrifuged for fifteen minutes were thirty-seven, fifty, and fifty-six per cent.

Centrifuging of the euglenae produced stratification of organelles similar to that illustrated by Patten and Beams in 1936,[2] and by Johnson in 1939.[3] As noted by these investigators, the paramylum bodies were forced to the

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[1]Mary Gojdics, The Genus Euglena (Madison, Wisconsin: The University of Wisconsin Press, 1953), p. 141; Jerome J. Wolken, Euglena (Rahway, New Jersey: Quinn and Boden Company, Inc., 1961), p. 7.

[2]Ruth Patten and Harold W. Beams, "Observations on the Effect of the Ultracentrifuge on Some Free-living Flagellates," Quarterly Journal of Microscopical Science, LXXVIII (July 1936), Pl. 30-31.

[3]Leland P. Johnson, "A Study of Euglena rubra Hardy 1911," Transactions of the American Microscopical Society, LVIII (January 1939), 45-46.

centrifugal pole, the chloroplasts and nucleus became somewhat centrally located, and the mitochondria were located at the centripetal pole. There was no apparent effect on the stigma and reservoir when the organisms were centrifuged for three and fifteen minutes. However, centrifuging for longer periods forced the stigma granules to the centripetal pole. The reservoir was forced to the periphery of the cell. The contractile vacuole was displaced only infrequently after centrifugation for fifteen minutes.

TABLE I

PERCENTAGE OF EUGLENA GRACILIS IN ELONGATED AND OVOID SHAPE BEFORE AND AFTER ULTRACENTRIFUGING

Cell shape	Centri. time	Before centri.	Immed. after centri.	Thirty min. after centri.	One hour after centri.	Two hours after centri.
Elong.	3 min.	70	32	51	54	55
Ovoid	3 min.	30	68	49	46	45
Elong.	15 min.	70	19	37	50	56
Ovoid	15 min.	30	81	63	50	44

The effect of cell centrifugation and the redistribution of organelles is illustrated in Table II. Percentages in Table II were based on five counts of ten for a total of fifty observations for each percentage listed. Random

samples were secured by moving the mechanical stage indiscriminately. The organism nearest the pointer tip was observed and recorded. In organisms centrifuged for three minutes, eighty-two per cent of the elongated forms and ninety-two per cent of the ovoid forms were stratified. Thirty minutes after centrifuging, redistribution of organelles was completed in fifty per cent of the elongated cells and in thirty-four per cent of the ovoid cells. After one hour, the organelles in sixty-six per cent of the elongated cells and sixty per cent of the ovoid cells were redistributed. Two hours following centrifuging, the organelles were redistributed in eighty-two per cent of the elongated cells and in sixty-eight per cent of the ovoid cells. Corresponding percentages for redistribution of organelles in organisms centrifuged for fifteen minutes were ninety, thirty-eight, sixty-two, and seventy-six for the elongated cells and ninety-six, twenty-eight, fifty-six, and sixty-six for the ovoid cells.

A variation in the per cent of organisms stratified occurred between those in elongated and ovoid states. A greater percentage of those in the ovoid state were stratified. Redistribution progressed at a slower rate in the ovoid cells. A higher percentage of stratification and a slower rate of redistribution of organelles was exhibited

in organisms centrifuged for fifteen minutes as compared to those centrifuged for three minutes. However, after two hours had elapsed, there was practically no difference in the per cent of organelle redistribution.

TABLE II

PER CENT STRATIFICATION AND REDISTRIBUTION OF ORGANELLES IN EUGLENA GRACILIS AFTER ULTRACENTRIFUGATION

Cell shape	Centri. time	Strati. immed. observ.	Redist. after thirty min.	Redist. after one hour	Redist. after two hours
Elong.	3 min.	82	50	66	82
Ovoid	3 min.	92	34	60	68
Elong.	15 min.	90	38	62	76
Ovoid	15 min.	96	28	56	66

With an increase in the time of centrifuging, the degree of layering of organelles became more pronounced. The layers of paramylum and chloroplasts were more compact at the centrifugal pole. The mitochondria became more concentrated at the centripetal pole. The stigma granules were also forced to the centripetal pole. On occasions, a vacuole was observed in the organisms centrifuged for fifteen minutes. In organisms centrifuged for longer periods of time, one or

more vacuoles were observed in the area between the chloroplasts and the mitochondria. Redistribution of organelles progressed at a much slower rate as the centrifuging time was increased.

Observations were made to determine what, if any, sequence was followed by the organelles in redistribution. These observations revealed that the nucleus was the first structure to return to its approximate position. This was followed by the chloroplast reorientation. The redistribution of the mitochondria and the paramylum bodies was completed at about the same time. In the stratified cell, the nucleus and chloroplasts occupied a more or less medial position while the mitochondria and paramylum bodies occupied the two extremes. In light of this, it seems likely that the sequence followed by the organelles in redistributing is dependent to a large extent on their relative positions in the stratified cell.

A comparison was made of the fixed and stained organisms with those in the free-living state. Observations of the prepared slides verified the information gained from viewing the living organisms.

The figures on page 21 illustrate a typical Euglena gracilis trophozoite--both free-living and fixed and stained Euglena, the effects of centrifuging, and various stages of

EXPLANATION OF FIGURE 1

Abbreviations: F, flagellum; R, reservoir; N, nucleus; P, paramylum body; C, chloroplast; M, mitochondrion; S, stigma; V, vacuole.

1. A typical *Euglena gracilis* trophozoite.

2. Stratification in an elongated cell centrifuged for three minutes.

3-5. Stages in redistribution of organelles in an elongated cell.

6. Stratification in an ovoid cell centrifuged for three minutes.

7. Cell centrifuged one hour.

8. Cell centrifuged one and one-half hours.

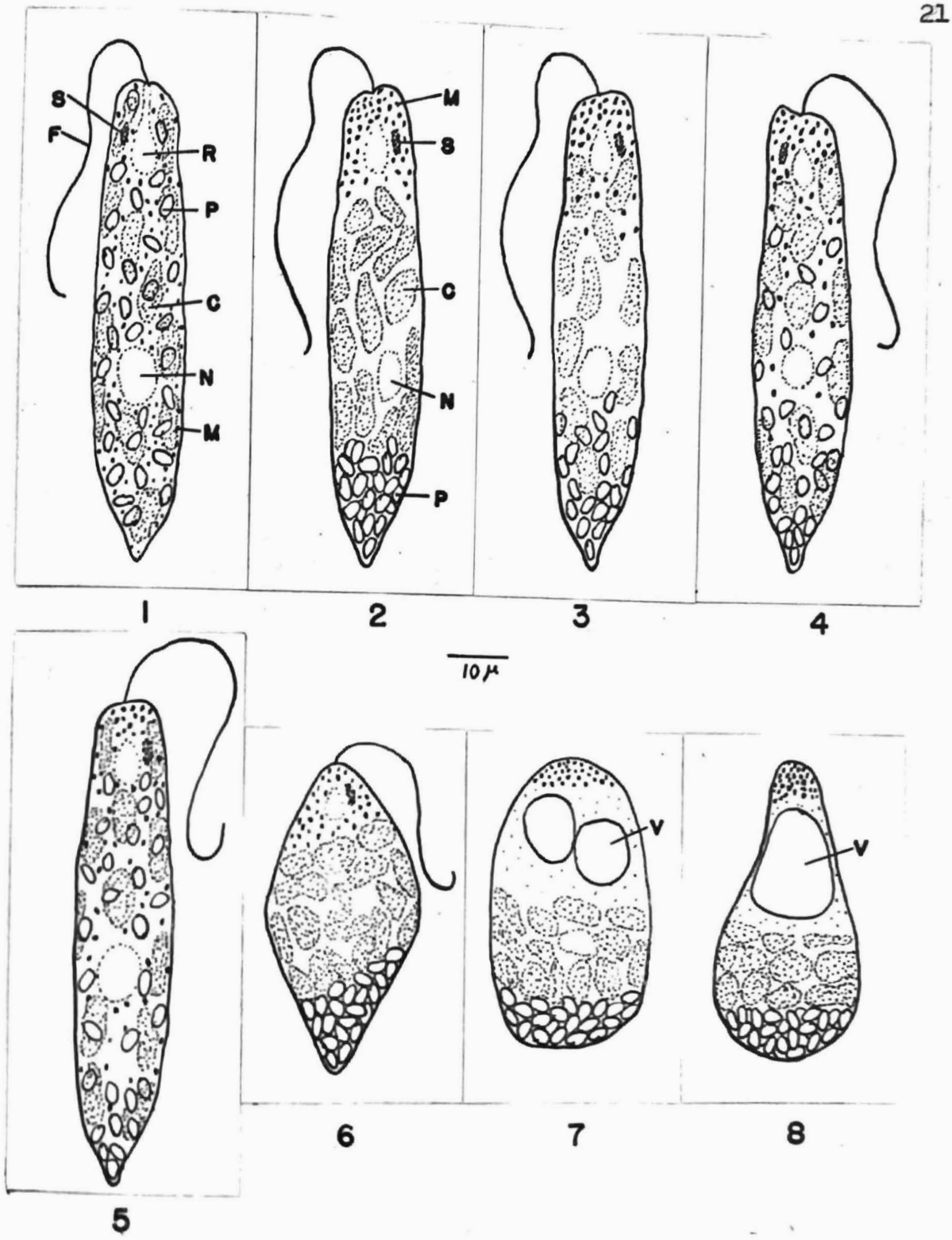

Figure 1. Protoplasmic constituents of Euglena gracilis before and after centrifugation.

organelles. The redistribution of organelles in the ovoid cell illustrated in number 6 of Figure 1 followed the same pattern as that in the elongated cell in numbers 3-5. A variety of shapes were found in the centrifuged cultures.

The appearance of vacuoles occurred frequently in organisms centrifuged for longer periods of time. After other organelles had redistributed, these were observed floating free at various points within the cell. Euglenae centrifuged for one and one-half hours had one large vacuole, located at the margin but centripetal to the chloroplasts. Since a vacuole contains water that is being eliminated from the cell, the relative positions of various organelles after centrifugation gave some evidence of comparative specific gravities of the organelles. Mitochondria and stigma granules were found centripetal to the vacuoles, while the chloroplasts and paramylum bodies were found centrifugal to the vacuoles. Mitochondria, predominantly fat-protein in make-up, are lighter than water presumably because of the large amount of lipid. Chloroplasts are also basically fat-protein; however, a greater concentration of protein must be present because they take a position after centrifuging which is associated with a specific gravity heavier than water. Paramylum bodies are carbohydrate in nature and possess a high specific gravity associated with their com-

pactness and centrifugal location. The presence of free-floating contractile vacuoles was observed in centrifuged Paramecium by King and Beams.[1] However, their ultimate fate was not determined. The vacuoles in Euglena were originally associated with the reservoir. By cytoplasmic movement the free-floating vacuoles could be transported to the anterior end of the organism and contact with the wall of the reservoir. The vacuolar membrane could fuse with the membrane of the reservoir and the contents of the vacuole eliminated, but this was not observed.

Observation of these vacuoles while the organisms were reorganizing revealed the presence of an unusual number of mitochondria at the surface of the vacuole. Whether or not these structures play a significant role in regulating water content in the cytoplasm requires further investigation.

Some unusual results occurred while conducting preliminary studies which deserve further attention. A patterned fluctuation occurred in the percentage of euglenae which maintained the elongated, active state. In organisms centrifuged for two and one-half hours and for three hours, more than fifty per cent were actively swimming. In organ-

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[1] R. L. King and H. W. Beams, "The Effect of Ultracentrifuging on Paramecium, With Special Reference to Recovery and Macronuclear Reorganization," Journal of Morphology, LXI (June 1937), 33.

isms centrifuged for two and three-fourths hours and three and one-fourth hours little locomotion was observed. Similar results were observed at three minutes, fifteen minutes, thirty minutes, and forty-five minutes. Few organisms were swimming after centrifuging for three minutes and thirty minutes. A large number of organisms were observed to be swimming following centrifuging for fifteen minutes and forty-five minutes. Were the organisms acclimated to the centrifugal force at various time intervals? This phenomenon was observed in mid-June when laboratory temperature was relatively stable and low. Attempts to duplicate these results later when external temperatures were high failed. Investigations along this line under carefully controlled conditions might prove rewarding.

In conclusion, the degree of stratification was determined by the length of time E. gracilis was exposed to ultracentrifugation. An increase in the time of exposure resulted in an increase in the time required for the organelles to become redistributed. The nucleus returned to its approximate normal position first; followed by the chloroplasts, mitochondria, and paramylum bodies.

CHAPTER V

SUMMARY

The study of the effect of centrifugal force on living cells has gained in importance in recent decades. The development of the air-driven ultracentrifuge enabled investigators to increase the amount of force applied far beyond the limits of mechanical devices.

Various cell organelles became stratified when the cell was subjected to centrifugal force. The purpose of this investigation was to study (1) the time required for redistribution of the various organelles of Euglena gracilis; and (2) what sequence, if any, was followed in the redistribution of organelles. The organelles observed were the nucleus, chloroplasts, mitochondria, paramylum bodies, and the stigma.

The cells were subjected to a force near 125,000 times gravity for periods of three minutes, fifteen minutes, thirty minutes, forty-five minutes, one hour, and one and one-half hours. The euglenae were also centrifuged for periods of time through three and one-fourth hours in preliminary studies. Microscopic examination was made of free-living organisms and fixed and stained organisms. Examination was made immediately after centrifuging to determine

the per cent of organisms stratified. Thirty minutes, one hour, and two hours after centrifuging, examination was made to determine the per cent of organisms exhibiting organelle redistribution completed and any sequence followed in the redistribution of these organelles.

Increase in the time the organisms were exposed to centrifugation resulted in increased stratification. The organelles in the euglenae which were centrifuged for three minutes were redistributed more quickly than those centrifuged for fifteen minutes. However, there was no significant difference between the two groups two hours after centrifuging. Organisms in the ovoid state had a higher percentage of stratification. Also, those in the ovoid state were slower in reorganizing.

There was a definite sequence followed by the organelles in redistributing. The nucleus was the first structure to return to its approximate normal position. This was followed by the chloroplasts, mitochondria and paramylum bodies. Relative position in the stratified cell was the apparent determining factor in the sequence followed.

Free-floating vacuoles were observed in organisms centrifuged for the longer periods of time. Though the eventual fate of these structures was not observed, it was reasoned by the investigator that these could eventually

fuse with the reservoir. Numerous mitochondria were observed at the surface of the vacuoles when the cell was reorganizing. Further investigation would be necessary to determine any significance attached to this.

In preliminary studies, a patterned fluctuation was observed in the percentage of actively swimming organisms after centrifuging. Subsequent attempts to duplicate these findings failed, when external temperatures were higher. Were the organisms acclimated to centrifugal force at various intervals? Further investigation under carefully controlled temperature might prove fruitful.

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